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Role of fructose in renal cell carcinoma progression

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Abstract
Renal cell carcinoma (RCC) is a highly malignant tumor with a poor prognosis, underscoring the urgent need for novel therapeutic strategies. RCC cells exhibit rapid proliferation and high metabolic demands, leading to hypoglycemic and hypoxic conditions within the tumor microenvironment (TME). Our study reveals that the fructose transporter Glut5 is prominently expressed in RCC, facilitating increased fructose uptake. This compensatory mechanism supports RCC survival under glucose deprivation and hypoxia. Fructose utilization sustains RCC proliferation, migration, and colony formation in vitro, significantly reduces apoptosis, and accelerates renal cancer growth in vivo. Mechanistically, fructose activates the cAMP/PKA signaling pathway, driving metabolic reprogramming and promoting tumor progression. Furthermore, 2,5-dehydro-D-mannitol (2,5-AM), a competitive inhibitor of fructose transport, significantly inhibits RCC growth both in vivo and in vitro. These findings provide new insights into the role of fructose metabolism in RCC progression and suggest potential therapeutic targets.

Keywords Renal cell carcinoma · Fructose · Tumor microenvironment · CAMP/PKA signaling pathway · Tumor progression

Renal cell carcinoma (RCC) is a malignant tumor with an increasing global prevalence. Approximately 400,000 new cases and nearly 175,000 deaths are reported annually worldwide, making RCC the leading cause of mortality among urinary system cancers [1]. Projections indicate that the incidence of RCC will continue to rise over the next decade, emphasizing the critical need to address this global health challenge. RCC is primarily classified into three subtypes: chromophobe renal cell carcinoma, papillary renal cell carcinoma, and clear cell renal cell carcinoma (ccRCC), with ccRCC accounting for approximately 70% of all cases [2]. RCC often remains asymptomatic until advanced stages, resulting in delayed diagnosis and missed opportunities for curative surgery. However, recent advances in biomedicine have introduced new drug treatments, such as Pazopanib for first-line therapy in intermediate-to-high-risk advanced RCC, and emerging targeted therapies like voronib [3–5]. Research on combining immunotherapy with targeted therapy is also advancing the development of new treatment strategies [6–8]. Despite these advancements, patient outcomes remain unsatisfactory due to drug resistance, severe side effects, and low efficacy [9, 10]. Therefore, identifying new therapeutic targets is crucial to improve patient prognosis.

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Since the discovery of the Warburg effect, the concept of metabolic reprogramming in tumors has gained recognition [11]. Tumor cells adapt their metabolic pathways to meet the demands of rapid growth and TME homeostasis. RCC cells, like many other tumor types, consume large amounts of glucose, leading to glucose depletion in the TME [12]. To compensate, tumor cells often utilize alternative carbon sources, such as fructose [13, 14]. Fructose, chemically similar to glucose, serves as a secondary energy source after glucose. Abnormal fructose metabolism is associated with various cancers, including lung adenocarcinoma, leukemia, pancreatic cancer, gliomas, triple-negative breast cancer, renal cancer, and prostate cancer [15–21]. Targeting fructose metabolism has shown promise in inhibiting liver metastasis in colorectal cancer, hindering lung cancer growth, and restraining tumor angiogenesis in liver cancer [22–24].

Glucose transporter 5 (Glut5), encoded by SLC2A5, is the primary fructose transporter [25]. It belongs to the SLC2A family of monosaccharide transporters and is expressed at low levels in most normal tissues, preventing direct fructose utilization [26]. In contrast, tumor cells express Glut5 and can metabolize fructose. Targeting Glut5 is an effective strategy to inhibit fructose metabolism. 2,5-dehydro-D-mannitol (2,5-AM), a fructose-based compound with tenfold greater affinity for Glut5 than fructose, specifically inhibits fructose absorption. Initially used for metabolic diseases, 2,5-AM has demonstrated antitumor effects and is now applied in preclinical studies for various cancers [15, 19, 27].

Research consistently shows that Glut5 expression and fructose utilization in tumor tissues correlate with tumor progression and patient outcomes [17, 28, 29]. Limited experimental research has investigated the link between targeting fructose metabolism and RCC progression. In this study, we present the first investigation into RCC cell progression characteristics under varying concentrations of fructose and glucose. We analyzed the advantages and disadvantages of fructose compared to glucose, the predominant sugar source for tumor cells. Additionally, we explored the functional relationship between fructose utilization and RCC progression. Our results demonstrate that fructose metabolism via Glut5 supports RCC cell viability, growth, and invasiveness in vitro and in vivo. Using 2,5-AM to inhibit fructose utilization produced therapeutic effects. Mechanistically, fructose drives RCC progression by modulating the cAMP-PKA signaling pathway, suggesting that targeting fructose metabolism could offer a promising therapeutic approach for RCC.

1 Cell lines and cell culture

The cell lines sourced from FuHeng Biology include human renal proximal tubular epithelial cells (HK-2) and RCC cell lines (SW839, 786-O, ACHN, 769-P, A498), as well as the mouse RCC line (Renca). SW839, 786-O, 769-P, and Renca were cultured in RPMI-1640 medium, ACHN in DMEM, A498 in MEM, and HK-2 in DMEM/F12 medium. Each culture medium was supplemented with 10% fetal bovine serum (Viva Cell) and 1% penicillin–streptomycin. Cells were incubated at 37 °C with 5% CO_2 and 21% O_2. Hypoxia was established using a 1% O_2 atmosphere. Dialyzed fetal bovine serum (DFBS), glucose-free RPMI-1640, glucose-free DMEM, glucose-free MEM, and glucose-free DMEM/F12 were obtained from Procell. We also used reagents including 2,5-am (41,107–82-8 Macklin) and H-89(S1643 Beyotime).

2 Cell proliferation assay

5000 cells were resuspended in 100 µL of different media and inoculated in 96-well plates, incubated at 37 °C for 72 h. 10 µL of CCK-8 reagent (NCM Biotech) was added to each well and incubated at 37 °C for 2 h. The absorbance at 450 nm was measured using an enzyme marker to assess cell proliferation and inhibition rates.

3 Colony formation assay

800 cells per well were incubated for 21 days in 6-well plates containing media with different sugars. After incubation, cells were fixed with 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet solution for 30 min. Images were collected for analysis.

4 Cell apoptosis detection

Cells were cultured in 6-well plates in different media for 72 h. Supernatant and digested cells were collected in 100 µL flow buffer with 10 µL propidium iodide and 5 µL Annexin V-FITC solution (Yeasen). Apoptosis analysis was performed using flow cytometry.

5 Wound healing assay

Cells were seeded in 6-well plates at densities allowing confluence the next day. Wounds were created using a 20 µL pipette tip, and different media were added. Cells were incubated at 37 °C, and images were recorded at 6, 12, and 24 h post-wounding. The wound healing rate was quantified using ImageJ software.

6 Migration assay

Transwell-24-well cell culture chambers with an 8 µm pore size were used. 2×10^4 cells were resuspended in 200 µL of glucose-free medium and placed into the upper chamber. Subsequently, 600 µL of medium supplemented with 10% fetal bovine serum was added to the lower chamber.

7 Immunofluorescence

Cells were incubated with a 1:200 dilution of primary antibody Glut5 (bs—3988R, Biosss) overnight at 4 degrees Celsius. Incubate with goat anti-rabbit IgG (H&L)—Alexa Fluor 488 secondary antibody (1:500) for 1 h at room temperature. DAPI staining was applied for 10 min to label cell nuclei. Images were captured using a Zeiss LSM 900 confocal fluorescence microscope, 488 nm (FITC) for detection of Glut5, 358 nm (DAPI) for nuclear visualisation.

8 Western blot analysis

Antibodies used in this study included anti-Glut5 (bs-3988R, Bioss), Na/K-ATPase (bs-1152R, Bioss), phosphorylated CREB-1 (bs-0036R, Bioss), rabbit antibody to CREB-1 (bs-0035R, Bioss), and GAPDH (6S-10900R, Bioss). All primary antibodies were diluted at a ratio of 1:800 and incubated overnight at 4 °C. Secondary antibodies were used at a dilution ratio of 1:10,000 and incubated for 1 h at room temperature. Protein bands were visualized using an ECL detection kit with picogram sensitivity.

9 Mouse xenograft model

Animal experiments were conducted according to the approved protocol from the Experimental Animal Center of Soochow University. Female BALB/c mice (4 weeks old) were obtained from Hangzhou Zhen Experimental Animal Technology. A total of 2×10^6 Renca cells were resuspended in serum-free RPMI-1640 medium and subcutaneously injected into the right axilla of the mice to establish subcutaneous tumor xenografts. Mice were randomly divided into four groups:

1. Control group: Fed distilled water and received intraperitoneal injections of saline (150 mg/kg/day).
2. 2,5-AM group (A888642-1, Macklin): Fed distilled water and received intraperitoneal injections of 2,5-AM (150 mg/kg/day) [23].
3. Glucose group: Fed 10% glucose water and received intraperitoneal injections of saline (150 mg/kg/day).

4. Fructose group: Fed 10% fructose water and received intraperitoneal injections of saline (150 mg/kg/day).

Body weight and tumor size were measured every two days. Tumor volume was calculated using the formula: Volume = Length × Width^2 / 2. Three weeks after cell transplantation, the mice were sacrificed by cervical dislocation. The experiment was approved by the Ethics Committee of Soochow University and conducted in strict compliance with institutional and national guidelines. Throughout the experiment, the tumor size/burden of all mice remained below the specified maximum.

10 Hematoxylin and eosin (H&E) and immunohistochemistry (IHC) staining of tissue sections

Tissue sections of mice were deparaffinized with xylene, dehydrated with graded alcohols, and stained with H&E. IHC staining was performed to quantify the expression levels of Ki67 (SAF008, Ai Fang Biology) and caspase-3 (AF06646, Ai Fang Biology) based on the percentage of positively stained cells.

11 Statistical analysis

Inter-group variance analysis was conducted using GraphPad Prism 9.0.0 software. Statistical tests included the Wilcoxon rank-sum test, Spearman's rank correlation coefficient, unpaired t-tests, one-way ANOVA, and two-way ANOVA. A p-value less than 0.05 was considered statistically significant.

12 Results

12.1 Glut5 expression is elevated in RCC and is associated with increased tumor burden

Immunohistochemistry (IHC) images from the Human Protein Atlas database (https://www.proteinatlas.org/) revealed that Glut5 expression was significantly higher in RCC tissues compared to normal renal tissue. Among the 10 cases of RCC tumor tissue IHC results presented, 8 cases showed moderate to high expression of Glut5 (Fig. 1A). The human kidney plays a central role in fructose absorption and metabolism. Glut5 is endogenously expressed in the microvilli of the proximal tubules of normal kidneys but at lower levels than in RCC tissues. RNA-seq data from RCC patients retrieved from the TCGA database (http://gepia.cancer-pku.cn/) consistently showed that both Glut5 mRNA and protein levels were markedly elevated in RCC tissues compared to normal kidney tissues (Fig. 1B). Furthermore, as Glut5 protein expression increased, tumor burden also increased significantly (Fig. 1C). To validate this observation, we selected one normal renal epithelial cell line (HK-2) and six RCC cell lines (SW839, 786-O, ACHN, 769-P, A498, and Renca) to assess Glut5 expression. Immunofluorescence and Western blot (WB) analysis confirmed that Glut5 expression was significantly higher in both human and mouse RCC cell lines than in normal renal epithelial cells (Fig. 1D and E).

12.2 Proliferation capacity of RCC cells in fructose is inferior to glucose at equal concentrations

We examined the impact of fructose on the in vitro viability of RCC cell lines, including SW839, 786-O, ACHN, 769-P, A498, and Renca. These cells were cultured in media containing different concentrations of sugars, including glucose-free media (devoid of both glucose and fructose), glucose (Glu) media, and fructose (Fru) media. Cell viability assays showed that fructose supported RCC cell survival in media containing 10% dialyzed fetal bovine serum (DFBS), with proliferation increasing at higher concentrations. However, normal HK-2 cells were unable to effectively utilize fructose for proliferation (Fig. 2A). Notably, at equal concentrations, fructose was less effective than glucose in promoting RCC cell proliferation.

To further evaluate proliferative capacity, we conducted colony formation assays. Based on proliferation results, we selected 786-O, which proliferated most actively in fructose, along with the relatively less active 769-P and the mouse RCC cell line Renca for further study. These three cell lines failed to form colonies when cultured in glucose-free media

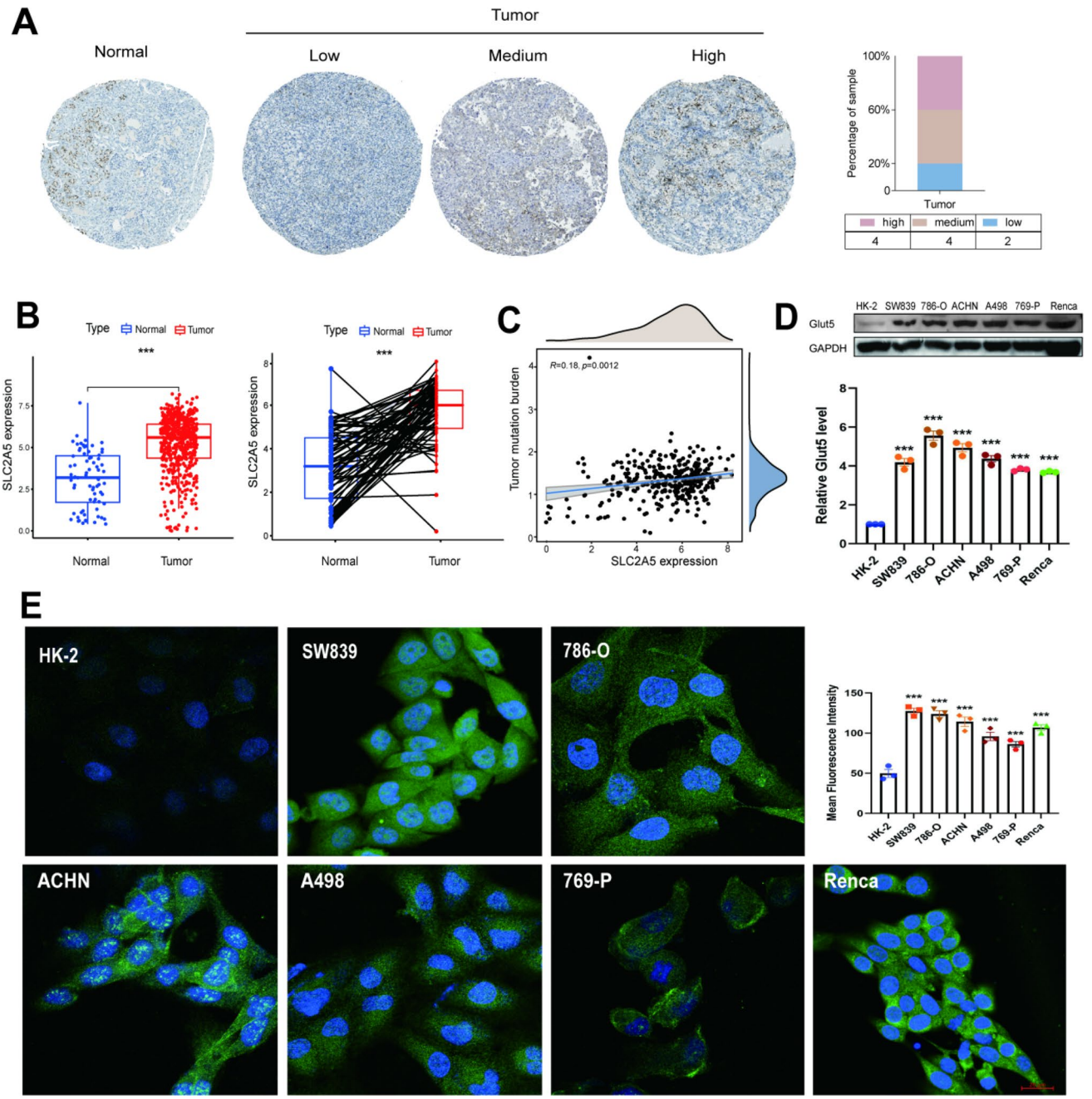


Fig. 1 Glut5 is Highly Expressed in RCC Cells **A** IHC images from the Human Protein Atlas database show that the majority of RCC tissues (8/10) exhibited moderate to high Glut5 expression. **B** Differential expression analysis of SLC2A5 in normal kidney tissues (n = 72) and ccRCC patients (n = 542) from the TCGA dataset. Paired differential analysis was performed using the Wilcoxon rank-sum test (**p < 0.001). **C** Correlation analysis of SLC2A5 expression with tumor burden in ccRCC patients using Spearman's rank correlation coefficient. **D** Western blot analysis and statistical evaluation of Glut5 expression in HK-2 and six RCC cell lines. Relative Glut5 levels are normalized to HK-2. **E** Immunofluorescence images and statistical analysis of Glut5 expression levels in HK-2 and six RCC cell lines. Error bars represent mean ± SEM. Data were analyzed by one-way ANOVA. *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$

supplemented with DFBS, whereas colony formation was observed in fructose-containing media (Fig. 2B and C). Consistent with the proliferation assay results, glucose was more favorable than fructose for colony formation. In summary, fructose can sustain RCC cell proliferation even when glucose is absent, while normal renal epithelial cells are almost completely dependent on glucose for growth and do not rely on fructose.

Fig. 2 Fructose Maintains Proliferation, migration and Inhibits Apoptosis of RCC Cells Cultured In Vitro. **A** RCC cell lines were cultured for 72 h with different fructose concentrations and oxygen conditions. The p-values were calculated by comparing cell proliferation in the presence of 0 mM fructose. **B**, **C** Colony formation images and statistical analysis of 769-P, 786-O, and Renca cells cultured in various sugar media. Cells were seeded at 1000 cells/well, and colonies were counted on day 14. **D** Fructose induces proliferation of RCC cells under different glucose conditions. The cells were cultured for 72 h in relevant media. The p-values for each glucose condition were calculated by comparing cell proliferation with that of the 0 mM fructose group. **E**, **F** Flow cytometric analysis of apoptosis was performed on 769-P, 786-O, and Renca cells, which were seeded at 2×10^5 cells per well in 6-well plates and cultured in different sugar media under hypoxic conditions for 72 h. **G** A Transwell assay was used to detect the migration ability of 769-P, 786-O, and Renca cells under different sugar conditions. **H**, **I** Wound healing analysis was performed on 769-P, 786-O, and Renca cells under different culture conditions. The control group was cultured in serum-free, glucose-free RPMI-1640 medium, and the other two groups were cultured in glucose-free RPMI-1640 supplemented with either 10 mM fructose or 10 mM glucose. Error bars represent mean ± SEM. Statistical analysis was performed using an unpaired Student's t-test for **A** and one-way ANOVA for **B**, **D**, **E**, **G**, **H**. * $p<0.05$; ** $p<0.001$; *** $p<0.0001$

12.3 Fructose significantly induces proliferation of RCC cells in glucose-deficient environments

We assessed whether fructose utilization could promote RCC cell proliferation under glucose-deficient conditions. Results showed that in low glucose (0.5 mM) or glucose-deprivation (0 mM) conditions, fructose significantly promoted RCC cell proliferation ($p<0.01$). When glucose was abundant (6 mM), additional fructose did not appear to be effectively utilized by the cells. However, under glucose deprivation (0 mM), even a small amount of fructose (0.5 mM) significantly enhanced proliferation (Fig. 2D). These findings indicate that when glucose is limited, fructose serves as an important alternative energy source for RCC cells, contributing more significantly to their proliferation under glucose-restricted conditions.

12.4 Fructose significantly reduces apoptosis of RCC cells under hypoxic conditions

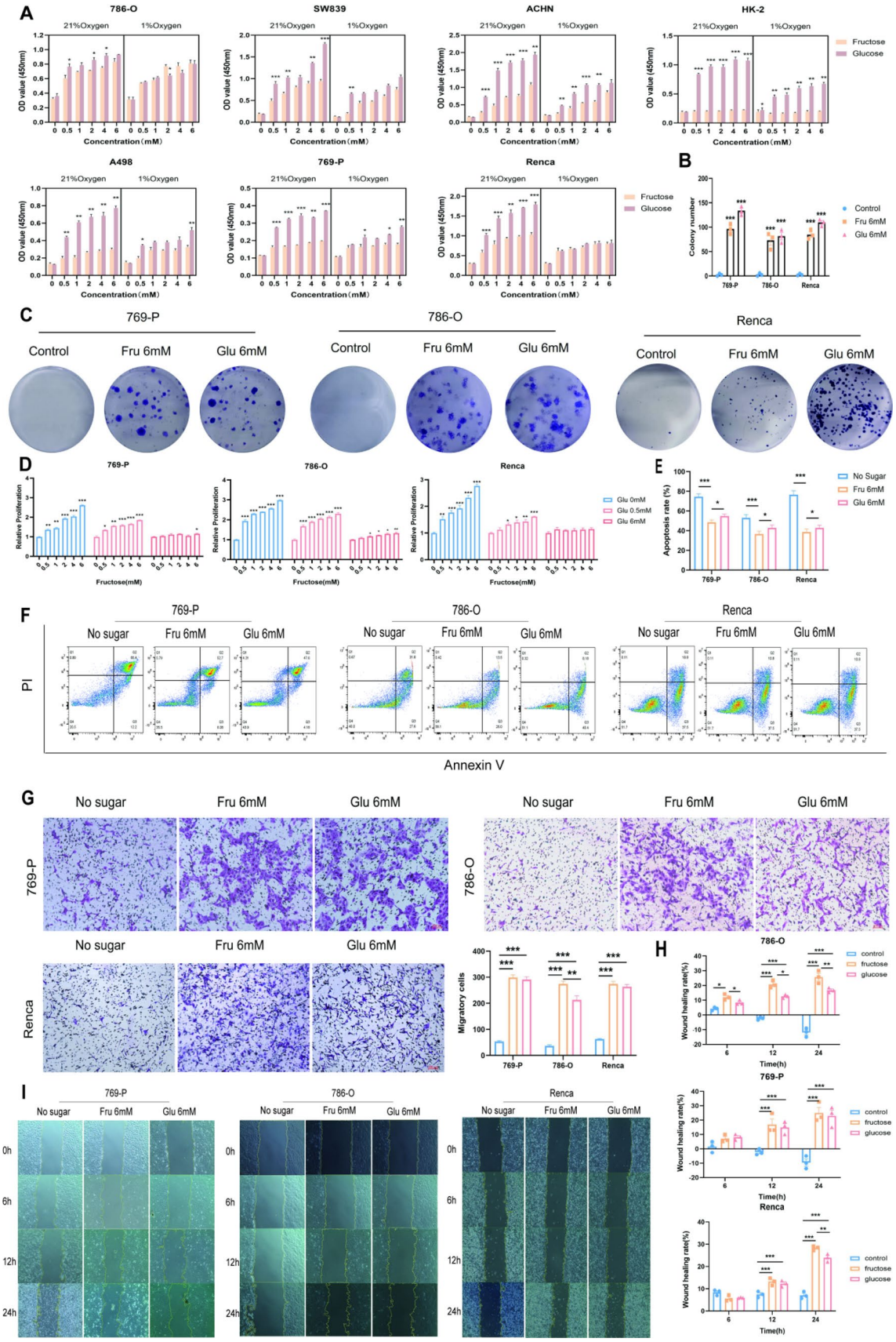
Hypoxia is a prominent feature of RCC, with oxygen levels gradually decreasing as tumor cells proliferate, leading to necrosis in central regions and hypoxic conditions in peripheral regions. Evaluating how fructose influences RCC cell survival under hypoxia is representative of the tumor microenvironment. RCC cells showed a difference in proliferation between fructose and glucose under normoxic conditions (fructose was less effective than glucose), but this difference narrowed under hypoxic conditions, with fructose reaching levels similar to glucose (Fig. 2A). In fact, RCC cell proliferation in fructose under both hypoxic and normoxic conditions was comparable, whereas glucose significantly reduced its proliferative capacity in hypoxia. Flow cytometry analysis confirmed that fructose substantially decreased apoptosis of RCC cells in hypoxia and demonstrated superior anti-apoptotic effects compared to glucose (Fig. 2E and F).

12.5 Fructose utilization leads to stronger RCC cell migration

Early metastasis is a hallmark feature of RCC. Our study found that RCC cells exhibited significant migratory ability in fructose-containing medium. The migratory capacity of 786-O cells was notably enhanced in the presence of 6 mM fructose compared to glucose, while 769-P and Renca cells showed similar migration abilities in both fructose and glucose media (Fig. 2G). Additionally, wound healing assays provided similar results. RCC cells were able to repair scratches using fructose as an energy source. In media containing equivalent concentrations of glucose and fructose, Renca cells showed no significant difference in wound healing ability. However, human-derived RCC cell lines, 769-P and 786-O, demonstrated stronger wound repair abilities in fructose medium (Fig. 2H and I). It is important to note that the control group of 769-P and 786-O cells in glucose- and serum-free medium showed significant cell death after 24 h, resulting in negative or undetectable wound healing rates at certain time points.

12.6 Enhanced fructose consumption promotes RCC cell progression in vivo

To assess how fructose intake influences RCC growth in animal models, our study randomly divided BALB/c mice subcutaneously inoculated with Renca cells into four groups: control group (normal water), 2,5-AM group (normal water), fructose group (10% fructose in water), and glucose group (10% glucose in water). Starting from the 15th day, in vivo tumor volume measurements showed a significant difference between the fructose-fed group and the control group (Fig. 3A). Notably, fructose intake significantly promoted the development of subcutaneous RCC tumors in mice. From the 15th day onwards, the tumor volumes of mice in the fructose and glucose groups increased rapidly in vivo, with


A
786-O
SW839
ACHN
HK-2
A498
769-P
Renca
21%Oxygen
1%Oxygen
OD value (450nm)
Concentration（mM）
Fructose
Glucose
B
Colony number
Control
Fru 6mM
Glu 6mM
769-P
786-O
Renca
C
D
Relative Proliferation
Fructose(mM)
Glu 0mM
Glu 0.5mM
Glu 6mM
E
Apoptosis rate (%)
No Sugar
F
No sugar
PI
Annexin V
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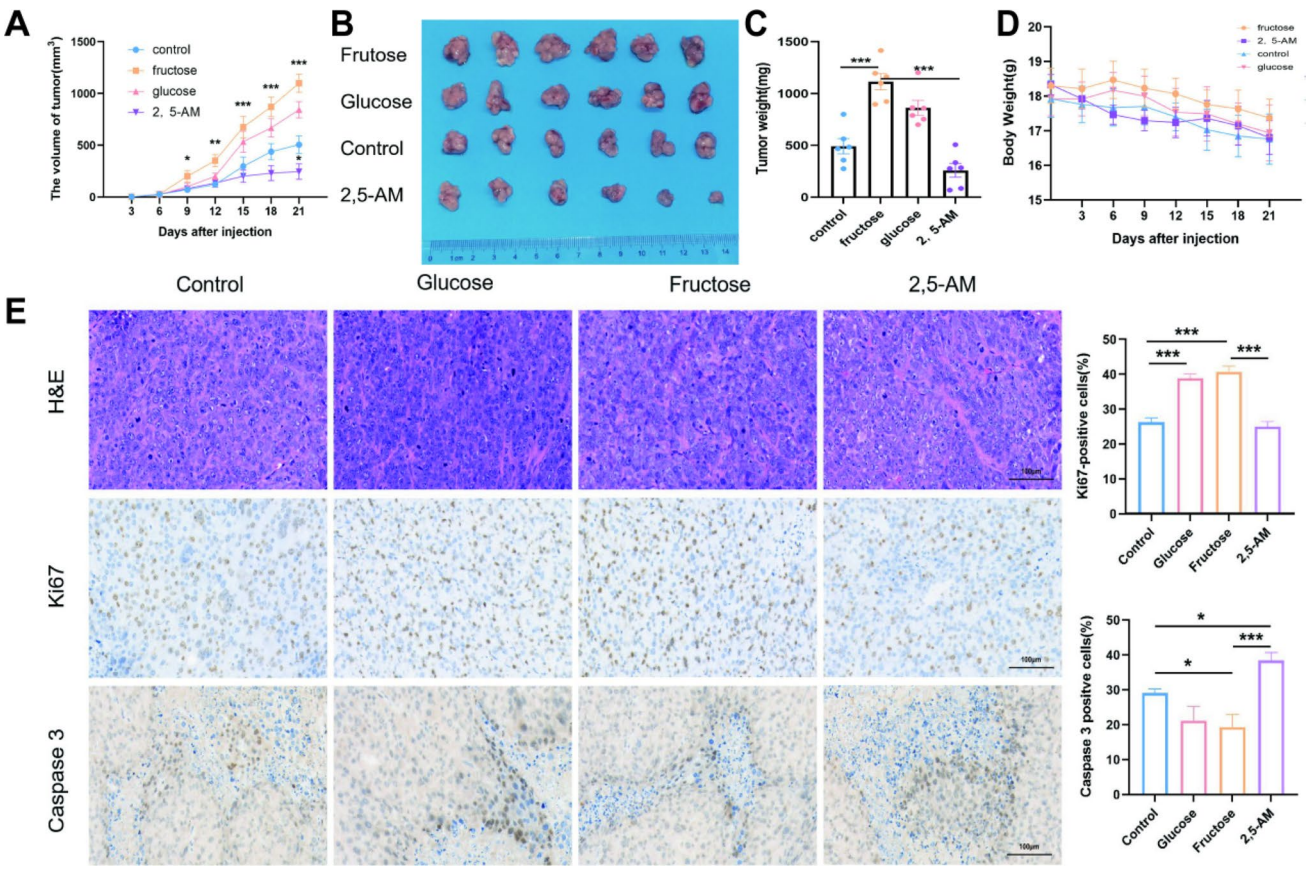


Fig. 3 Fructose Accelerates the Growth of Subcutaneous Tumors In Vivo. **A** Changes in tumor volume over time after subcutaneous tumor implantation. **B**, **C** Resected tumor tissues and weights of mice in four groups. **D** Variations in body weight among the mice in each group after tumor-bearing. **E** Photographs (×400) of H&E and IHC staining, as well as statistical results of Ki67 and caspase-3 expression in tumor samples. Error bars represent mean ± SEM. Data were analyzed using one-way ANOVA (**B**, **E**) or two-way ANOVA (**C**, **D**). * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

more significant differences compared to the control group. The tumors harvested from the fructose-fed group had larger volumes and weights than those from both the control and glucose groups (Fig. 3B and C). The body weights of the mice in all four groups decreased slightly over time after inoculation, but this change was not statistically significant across the different groups. IHC results, including Ki67 and caspase-3 staining, showed increased tumor proliferation and decreased apoptosis in the fructose group (Fig. 3E). These findings indicate that fructose plays a crucial role in promoting RCC proliferation.

12.7 2,5-AM remarkably suppresses RCC cell proliferation in animal models and cell cultures

The cell proliferation-toxicity assay showed that 2,5-AM effectively suppresses the proliferation of RCC cells in the presence of fructose. However, the proliferation of RCC cells in glucose was not inhibited with increasing doses of 2,5-AM. This indicates that 2,5-AM is both effective and specific in inhibiting fructose uptake by RCC cells in vitro (Fig. 4A). Colony formation assays on 786-O, 769-P, and Renca cells demonstrated that in the presence of 2,5-AM, the colony-forming capacity of RCC cells in fructose-containing medium was significantly reduced. At a concentration of 1 mM 2,5-AM in 6 mM fructose medium, most RCC cell colonies were inhibited, and at 2 mM, hardly any RCC cells could survive (Fig. 4B). In animal experiments, on the 21st day after tumor implantation, there was a significant difference in tumor volume between the 2,5-AM group and the control group. While 2,5-AM did not prevent the initial growth of RCC cells, it significantly inhibited continued tumor growth. Both the volume and weight of tumors in the 2,5-AM group were significantly smaller compared to the control, glucose, and fructose groups, indicating that 2,5-AM shows a relatively good therapeutic effect in treating RCC in vivo.

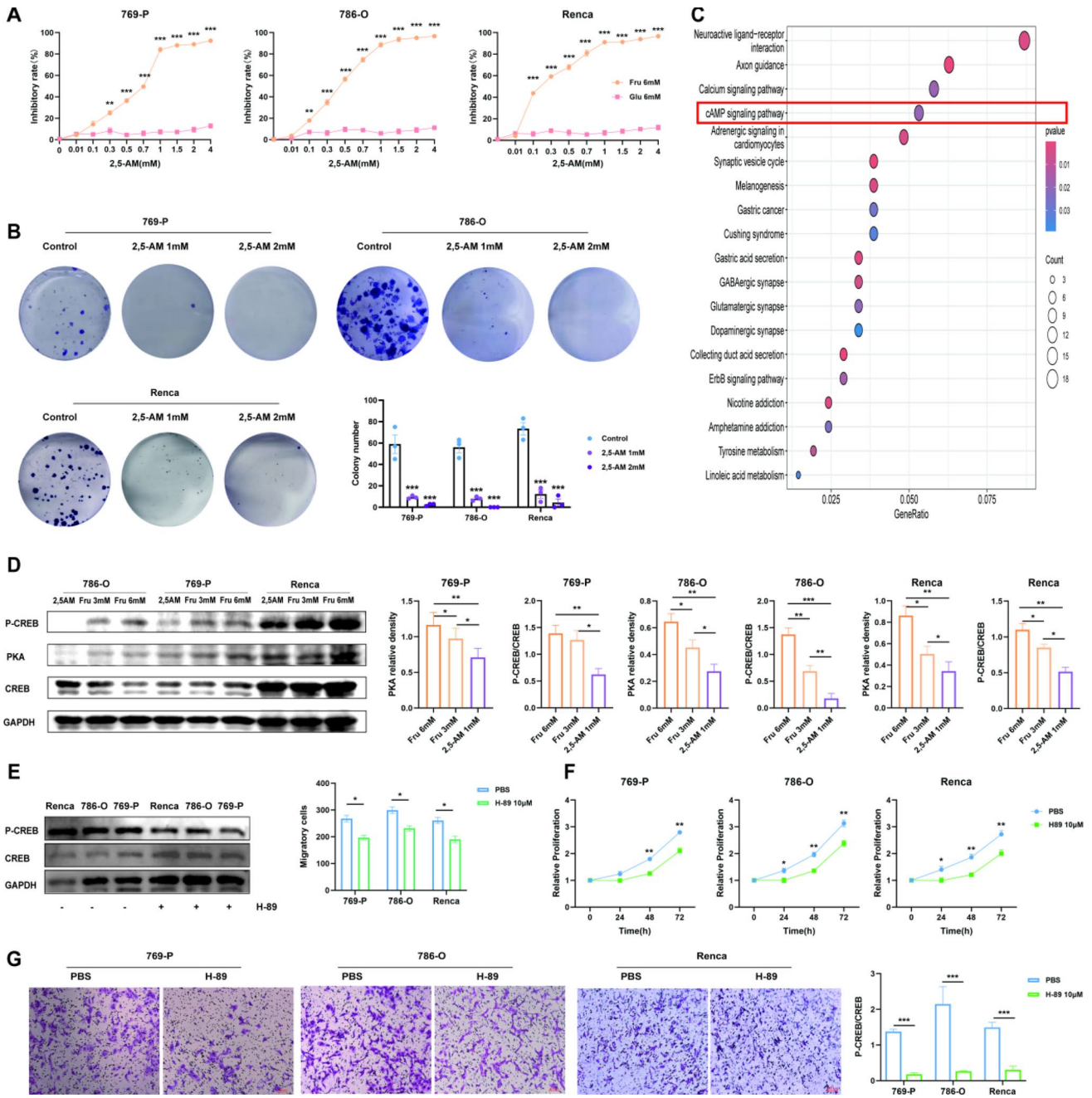


Fig. 4 2,5-AM Suppresses RCC Cell Proliferation. **A** Inhibition of RCC cells after treatment with different concentrations of 2,5-AM. **B** Colony formation and statistical analysis of 769-P, 786-O, and Renca cells in 6 mM fructose under 2,5-AM intervention for 2 weeks. **C** KEGG enrichment analysis of signaling pathways in RCC cells involving SLC2A5. **D** Western blot verification of the activation status of the cAMP/PKA signaling pathway in 769-P, 786-O, and Renca cells under different intervention conditions, 2,5-AM group refers to the addition of 1 mM of 2,5-AM to 6 mM fructose medium,with statistical analysis. RCC cells containing 6 mM fructose medium were treated with PBS or H-89 10 μm for 48 h. Cells collected were used for Western blot (**E**) and proliferation assays (**F**) and migration assays (**G**). Error bars represent mean ± SEM. Analysis was performed using unpaired Student's t-test for **A**, **E**, **F**, **G** and one-way ANOVA for **B**, **D**.* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

12.8 Fructose utilization in RCC Cells activates the cAMP/PKA pathway

We conducted bioinformatics analyses to investigate potential signaling pathways related to tumorigenesis. Through differential expression analysis, we identified genes significantly upregulated in tumor tissues and used KEGG pathway enrichment analysis to discover multiple signaling pathways involved in cell proliferation and apoptosis (Fig. 4C).

Among these pathways, the cAMP signaling pathway ranked highly, suggesting that fructose utilization might play a crucial role in RCC development through this mechanism. To confirm the activation of the cAMP/PKA signaling pathway in RCC, we used Western blot (WB) to detect the phosphorylation status of related proteins. WB results showed that, compared to the fructose concentration of 3 mM, the phosphorylation density of p-Akt and p-mTOR in three types of RCC cells (769-P, 786-O, and Renca) cultured in experimental groups with increased fructose was significantly increased. Conversely, in the 2,5-AM inhibition group, the expression levels of P-CREB and PKA decreased significantly, further confirming the activation status of the PI3K/Akt signaling pathway in tumor cells (Fig. 4D). We standardized protein loading amounts in each group using total CREB protein and GAPDH as internal reference proteins to ensure result reliability.The next step is to perform a rescue assay with H-89, a PKA inhibitor, to validate the role of the cAMP/PKA pathway.As expected, H-89 inhibited the upregulation of P-CREB (Fig. 4E), as well as the proliferation (Fig. 4F) and migration of the three RCC cells (Fig. 4G).

13 Discussion

Tumors exhibit high metabolic activity, leading to severe nutrient deficiencies. The lack of carbohydrates, which are crucial energy sources for tumors, is particularly pronounced. Consequently, tumors undergo metabolic reprogramming to utilize alternative carbon sources. Many tumor tissues upregulate the Glut5 protein to enhance fructose utilization. Higher Glut5 expression in tumors often correlates with a more aggressive malignant phenotype.

Fructose ingested into the body is absorbed through Glut5 in the small intestinal epithelial cells and transported to the liver via the portal vein [30]. There, fructokinase metabolizes it directly into fructose-1-phosphate without insulin regulation. Subsequently, it is converted into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate through a series of enzymatic reactions. These intermediates can enter the glycolytic pathway but bypass the glucose-6-phosphate step [31]. Unlike glucose metabolism, fructose lacks the feedback inhibition mechanism of glucose-6-phosphate, allowing it to rapidly supply energy for tumors.

Previous data from the TCGA database indicated that Glut5 was a poor prognostic factor for RCC. However, after the April 2024 update, Glut5 became a favorable prognostic factor for ccRCC. Validation in the Human Protein Atlas database has not reached a consistent conclusion, highlighting the need for further investigation. Our research confirms that fructose is a critical nutrient that RCC cells can uptake and process, thereby promoting RCC progression. Fructose metabolism serves as a crucial adaptive mechanism for RCC cells, especially under hypoxic and glucose-scarce conditions within the tumor microenvironment.

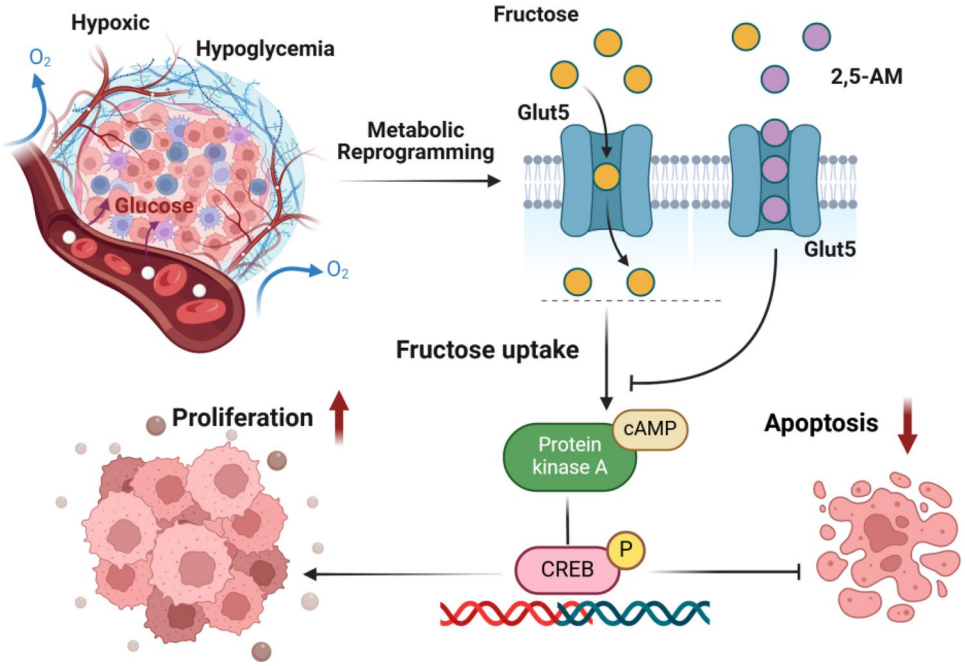
Research on fructose and various cancers reveals a contradictory phenomenon. While fructose at the same concentration is less effective than glucose in promoting cell proliferation in vitro, animal experiments often show that the tumor proliferation rate in the fructose group approaches or even exceeds that of the glucose group. As a backup carbohydrate energy source, the role of fructose in tumor progression cannot be simply judged by its proliferation ability alone.

The tumor microenvironment is characterized by low sugar and low oxygen levels. Our research shows that under these conditions, RCC cells significantly increase their utilization of fructose and notably inhibit apoptosis. Moreover, fructose contributes more to tumor migration than glucose.

Previous studies have shown that fructose metabolites can induce vascular endothelial growth factor expression, promoting angiogenesis and supporting tumor growth and metastasis [32]. Additionally, fructose can inhibit T-cell and natural killer cell functions, trigger polarization of tumor-associated macrophages, and produce pro-tumor factors by regulating tumor microenvironment acidification or promoting inflammation, thereby reducing the immune system's ability to recognize and eliminate tumors [33–35]. However, some studies have found that dietary fructose can regulate adipocyte metabolism to enhance the anti-tumor immune response of $CD8^+$ T cells and control lung cancer growth [36]. Clearly, the relationship between fructose metabolism and anti-tumor immunity requires further in-depth research.

Research on targeting sugar metabolism indicates that inhibiting glucose metabolism has potential as an anti-tumor treatment but also has significant side effects [37–39]. These side effects primarily affect normal cells and organs, particularly damaging systems such as the nervous, immune, liver, and heart systems. Issues like lactic acid accumulation, metabolic imbalance, malnutrition, and changes in gut microbiota require key attention [40].

On the other hand, disorders in fructose utilization can lead to increased circulating fructose and fructofuranose. No adverse reactions such as increased blood insulin or elevated diabetic complications have been reported in human and animal experiments [19, 41, 42]. Glut5 is a protein containing 12 transmembrane helices that transports fructose via facilitated diffusion [43]. It has a low affinity for glucose and does not participate in glucose transport



Scheme 1 Glut5-dependent fructose metabolism activates RCC cell progression through the cAMP/PKA pathway

[44]. In our research, the fructose competitive inhibitor 2,5-dehydro-D-mannitol (2,5-AM) had minimal impact on glucose. Previous studies have demonstrated that 2,5-AM has therapeutic potential to inhibit tumor cell proliferation and metastasis, suppress lactic acid production from Glut5-mediated fructose utilization, and produce a significant synergistic effect when combined with chemotherapeutic drugs, without obvious side effects. In our current animal experiments, 2,5-AM showed a therapeutic effect on RCC. Additionally, the body weights of mice in the 2,5-AM group did not differ significantly from those in the other groups, indicating no severe adverse reactions from the 2,5-AM injection during the experiment. Although the mice in the 2,5-AM group did not have fructose added to their diet, the body has multiple metabolic pathways, such as the polyol pathway, that can produce endogenous fructose [45, 46]. Through their own metabolic activities, a certain amount of fructose can be provided for tumor cells from other substrates. 2,5-AM can significantly inhibit the uptake of fructose by tumor cells, thereby limiting the promoting effect of fructose on RCC. Targeting fructose treatment for RCC using 2,5-AM appears to be an effective and mild approach.

The cAMP signaling pathway is a critical intracellular signaling route essential for various physiological processes [47]. In renal cells, the cAMP signaling pathway regulates intracellular metabolic processes and maintains the balance of water and electrolytes. By activating protein kinase A (PKA), cAMP affects the permeability and transport efficiency of ion channels, ensuring normal kidney functions such as filtration and reabsorption [48]. In RCC, the cAMP signaling pathway influences tumor cell proliferation. Studies have shown that intracellular cAMP levels are abnormally elevated in some RCC cells, disrupting normal cell proliferation regulation and promoting rapid tumor growth [49]. The cAMP signaling pathway also plays a key role in regulating apoptosis in RCC [50]. Dysregulation of this pathway can lead to increased expression or inhibition of anti-apoptotic proteins, enabling cancer cells to evade normal apoptotic clearance mechanisms [51].In this study, the activation of the cAMP/PKA signaling pathway became more evident with increasing fructose concentration, and 2,5-AM clearly inhibited this pathway. This indirectly indicates that fructose utilization directly activates the cAMP/PKA signaling pathway. The fact that RCC activates the cAMP/PKA signaling pathway through fructose utilization explains the increased proliferation and decreased apoptosis observed in our phenotypic experiments (Scheme 1).

The 6 mM sugar concentration used in this study closely mimics the physiological blood glucose concentration in adults. This concentration ensures cellular metabolism without causing metabolic abnormalities due to overly high or low concentrations. Meanwhile, three types of RCC cells were used for in-depth research to prove the universality of the results. Our findings show that Glut5 is highly expressed in RCC, providing a crucial survival mechanism within the hypoxic and glucose-scarce tumor microenvironment. In vitro, fructose sustains RCC proliferation and migration. It significantly promotes cell proliferation under low-glucose conditions and effectively inhibits RCC cell apoptosis in hypoxic environments. In animal experiments, fructose consumption promotes faster RCC growth in mice compared to the glucose group. Fructose utilization in RCC activates the cAMP/PKA signaling pathway, thereby promoting tumor progression.

RCC is essentially a metabolic disease characterized by the reprogramming of energy metabolism, and intermediates of the glycolytic pathway may be preferentially used in the synthesis of biomacromolecules to meet the needs for rapid proliferation of tumor cells [52–54]. The fructose metabolism, explored in this study, has a tight link with glycolysis. After entry into cells via the transporter Glut5, fructose can be converted into glycolytic intermediates through a series of enzymatic reactions, such as glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, thus entering the glycolytic pathway and providing additional energy source for tumor cells. At the same time, because mitochondrial bioenergetics and oxidative phosphorylation (OxPhox) function in RCC patients are impaired, tumor cells may further rely on anaerobic metabolic pathways such as glycolysis and fructose metabolism to maintain their energy needs, and upregulate the expression of Glut5 through tumor cells, thus enhancing the uptake and utilization of fructose [55]. Moreover, the lipid metabolism is also abnormal in RCC [56, 57]. Acetyl-CoA produced by fructose metabolism can promote lipid synthesis and cell signaling pathway regulation in tumor cells, supporting the proliferation, invasion, and metastasis of tumor cells. Therefore, a deep understanding of the complex interrelationships between fructose metabolism, glycolysis, mitochondrial function, and lipid metabolism in RCC is important for the development of more effective therapeutic strategies. Future studies could further explore how to achieve a more precise and effective treatment of RCC by intervening in the interaction between these metabolic pathways.

Author contributions Jixuan Miao, Xingwei Sun and Jin Yong contributed equally to this work. Jixuan Miao, Xingwei Sun and Jin Yong designed the project. Jixuan Miao, Xingwei Sun, Yuyun Wu, Di Wang, Ruochong Pang and Hao Zhang contributed to the characterization, performance experiments, and biological experiments. Jixuan Miao, Xingwei Sun and Jin Yong contributed to the writing, review, and editing of the manuscript. Xingwei Sun and Jin Yong contributed to the supervision and project development.

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Declarations

Consent for publication All authors agree to publish.

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